Combination of electrospun nanofibers and embryonic stem cells for nerve injury repair

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Statement of Purpose: Embryonic stem (ES) cells are pluripotent cells, which have the capacity for continuous self-renewal. Considerable attention has focused on the role of ES cells or their derivatives in the repairing of nerve injury. It has been demonstrated that the differentiation of ES cells into motor neuron and oligodendrocytes could be induced using various chemical cues.[1] Previous studies clearly established that transplantation of ES cells could be used for nerve repairing including both central and peripheral nervous systems.[2, 3] Electrospinning is widely employed to fabricate nanofibers for various biomedical applications. The fiber diameter and composition can be easily controlled. Electrospun nanofibers can also be functionalized either by blending, encapsulation, or immobilization of bioactive materials to elicit specific biological responses. Furthermore, electrospun nanofibers can be aligned uniaxially with anisotropic properties and they can be utilized to construct microstructured units such as sheets, disks, and tubes. A combination of ES cell therapy and nanofibrous scaffold could provide a better strategy for nerve injury repairing. The objective of present study is to investigate embryonic stem cells seeded electrospun nanofibers for nerve injury repair.

Methods: The electrospinning setup and the collector used for fabricating and collecting aligned nanofibers are similar to those used in our previous studies.[4, 5] The polymer solution used for electrospinning contained 20% PCL (w/v) in a mixed solvent of dichloromethane and dimethylformaldehyde with a volume ratio of 80:20. Two different collectors -- a cover glass and a stainless steel frame with an air gap -- were employed to collect randomly and uniaxially aligned fibers, respectively. The aligned fibers were transferred to a glass cover slip and then fixed by medical grade silicon adhesive. The PCL fibers were sputter-coated with gold before imaging with scanning electron microscope. Immunohistochemistry was performed to visualize the spatial distribution of cells and neurites

Results: To investigate the topographic effect on the differentiation of ES cells, aligned and random PCL nanofibers were fabricated using electrospinning. The aligned nanofibers were obtained using a metal frame with a void gap as a collector, which was developed by our group.[4, 5] The fiber diameter was around 250 nm. It is found that cells that had migrated out of the EBs stained positive for Tuj1 and possessed axon-like processes. However, neurites grew along all the directions surrounding the EB's main body. Also, oligodendrocytes and astrocytes migrated randomly to the surrounding region of EBs (data not shown). Tuj1 staining is localized to cells which display a clear neuronal phenotype, suggested by the cell body with extending, axon-like protrusions. Interestingly, neurites grew along the direction of nanofiber alignment. Moreover, oligodendrocytes migrated along the direction of aligned fibers, indicating possible myelination around the axons of the neurons (data not shown).

To restore function within degenerating regions of the nervous system, transplanted embryonic stem cells must differentiate and extend axons that form synaptic contacts within the host tissue. Here, we also developed the EBs-seeded conduit fabricated by rolling electrospun nanofibers. Fig. 1A shows the schematic of the EBs-seeded conduit with a tubular shape composed of random nanofibers at two ends and aligned nanofibers in the middle. The random fibers at the ends could provide sufficient mechanical strength for suture. CE3 EBs (CE3 expresses green fluorescent protein) were seeded inside conduit and cultured for 14 days. Fig. 1B shows that CE3 EBs were seeded inside the conduit. Fig. 1C shows the Tuj1 staining of EBs seeded inside the conduit. It is observed that many cells extended neurites. The inset image suggested neurites outgrowth along the direction of nanofiber alignment, which could possibly connect the gap and be helpful for signal transduction.

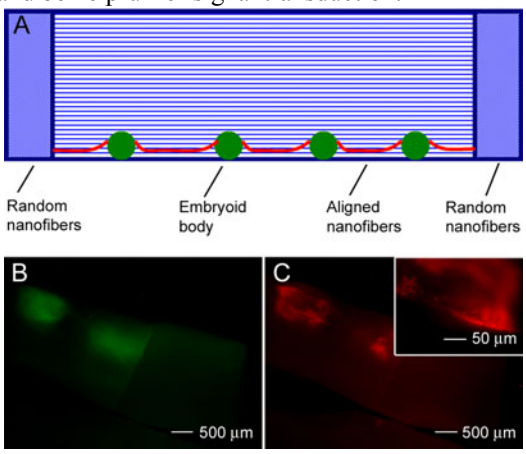

Figure 1. A) Schematic illustrating the conduit composed of random, aligned electrospun nanofibers and embryoid bodies. B) EB-seeded conduit (green color owing to CE3 cells expressing GFP). C) Tuj1staining (a neuron marker). Inset: higher magnification, suggesting neurites extension along the direction of fiber alignment.

Conclusions: We have demonstrated that ES cells could differentiate into neural lineages. Aligned nanofibers could not only enhance the differentiation into neural lineages but also direct the neurite outgrowth. The present study may provide a new platform that combines nanostructured scaffolds and ES cell therapy for nerve injury repair.

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